SCIENTIFIC PAPER

Topical Treatment With Oxaliplatin for the Prevention of Port-Site Metastases in Laparoscopic Surgery for Colorectal Cancer

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ABSTRACT

Background: The development of port-site metastases following laparoscopic resection of various malignancies continues to be a disturbing issue for laparoscopic surgeons. Previous studies revealed promising results with oxaliplatin, a third-generation platinum compound, as a first-line treatment in advanced colorectal cancer. This study evaluates the effect of topical application of oxaliplatin on the development of port-site metastases in an experimental murine model.

Methods: Nineteen female BDIX rats (immunocompetent, 6 weeks old) underwent a sham laparoscopic operation after $1x10^7$ viable rat colon carcinoma viable cells (LMCR) had been injected into their peritoneal cavities. Three trocars (1 central camera port and 2 additional lateral ports) were introduced into the abdomen, and a pneumoperitoneum was created with carbon dioxide. Ten minutes after LMCR, cells were injected into the peritoneal cavity, the 2 lateral trocars were removed and carbon dioxide insufflation was maintained for an additional 5 minutes to allow for tumor cell seeding. Oxaliplatin (0.198 mg/kg) was then topically applied to 1 trocar site intramuscularly, while the other site was left untreated. One week later, the animals were euthanized, and the port sites were histologically examined for evidence of metastases.

Results: The rate of tumor implantation at the muscle layer in control sites was 68% (13/19) compared with 37% (7/19) at oxaliplatin-treated sites (P=0.1). Also, no significant differences were detected in port-site metastasis rates in other untreated layers of the abdominal wall.

Conclusion: Intramuscular topical application of oxaliplatin may not decrease the incidence of port-site metastasis in a syngeneic animal model of colon cancer. Nevertheless, we can see the tendency of declination. Further studies are needed to better determine its possible therapeutic role in high-risk humans undergoing laparoscopic resection of colorectal malignancies.

Key Words: Laparoscopy, Oxaliplatin, Wound, Metastasis, Colorectal cancer, Port-site metastases.

INTRODUCTION

Laparoscopic surgery for malignancy continues to be a matter of contention. While in the beginning of the laparoscopic era, the major concern was focused on technical feasibility, it was not until several reports describing port-site recurrences appeared,[1–4] that the applicability of laparoscopy in these cases was questioned. Multiple publications have demonstrated the advantage of laparoscopy over open surgery regarding postoperative recovery and better cosmetics[5–7]; however, questions as to adequacy of resection, long-term oncological outcome and port-site recurrences, limited its application in cancer surgery. Recently, several studies showed that the oncological results are, at least, the same as in open surgery, and surprisingly the rates of port-site metastasis were comparable.[7–10] The real incidence of this particular way of tumor spreading is not known, nor is its prognostic implication. It seems that in experienced hands the incidence does not significantly differ from that in open surgery.[7,9,10] However, since the greater bulk of procedures is not performed by highly skilled laparoscopic surgeons, this continues to pose a problem that should be addressed during surgery. Nonetheless, even though its occurrence is considered low (1.1% to 3.9%),[11,12] overall it is still higher than what is expected for open surgery (0.6% to 0.8%).[11]

The mechanisms proposed for this unwarranted phenomenon include excessive manipulation of the tumor, CO_2 insufflation, air leakage through port sites (desufflation), direct implantation with contaminated instruments, and contamination while extracting the specimen.[13] However, the precise mechanism is not known yet. Nonetheless,

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while most of these causes can, theoretically, be prevented through better surgical technique and experience, it has been noted that tumor cells are being spilled in almost half of the patients undergoing open cancer surgery.[14] This fact has drawn the attention of several investigators, who published a number of articles addressing this issue.[11,13,15–18] Some therapeutic agents have been tested including Povidine Iodine and 5-FU, which showed significant potency in preventing tumor growth at port sites,[11,16,17,19] but none displayed a total protection. In those studies, intraperitoneal irrigation or systemic administration of cytotoxic agent might prevent tumor implantation after laparoscopic surgery and port-site metastases. The use of intraperitoneal heparin also can prevent tumor implantation by reducing the presence of intraperitoneal blood.[18]

On August 2002, the FDA approved oxaliplatin for the treatment of colorectal cancer in those patients refractory to 5-FU and Irinotecan. This new drug has been demonstrated to be effective in the treatment of patients with colorectal cancer.[20] The aim of our study was to evaluate the efficacy of this new cytotoxic drug as a topical treatment to prevent port-site metastasis following laparoscopic surgery for colorectal cancer.

METHODS

Cell Cultures and Animals

A metastatic rat colon carcinoma cell line (LMCR) with Sialyl-Tn (STn) negative clones was used in this study. The cell line was originally derived in the Gastrointestinal Research Laboratory, Department of Medicine, Mount Sinai School of Medicine.[21] The cell line was grown in DMEM supplemented with 10% fetal calf serum, 50 units/mL penicillin, and 50 μg/mL streptomycin and incubated at 37°C in 7.5% CO_2. Nineteen female BDIX rats (4 to 6 weeks old) obtained from Charles River Laboratories through the National Cancer Institute were used. This number of animals will allow detection of a difference of 50% in the incidence of port-site metastasis (80% for control group and 30% for each group)[11] with power 0.80 for a 2-sided test at the 0.05 level of significance. Our Institutional Animal Care and Use Committee approved the study.

Cytotoxicity Assays

To determine the optimal concentration of oxaliplatin for use in the animal experiment, the oxaliplatin concentration that inhibits 50% of cell growth (IC_{50}) was determined by using an MTT (3, 4, 5-dimethylthiazool-2, 5-diphenyltetrazolium bromide; Sigma) assay. $4x10^3$, $8x10^3$, and $1.2x10^4$ cells/well were seeded into a 96-well plate, which was incubated for 24 hours. The cells were then treated with various concentrations of oxaliplatin, and incubated for an additional hour at 37°C. Subsequently, 10 μL of MTT at a concentration of 5 mg/mL was added to each well and cells were incubated for an additional 4 hours to 6 hours. The supernatant was aspirated and 100 μL of dimethylsulfoxide was added to the wells to dissolve any precipitate present. The optical density was then measured at a wavelength of 570 nm by using an ELX800 plate reader (Bio-Tek Instruments, Inc., Winooski, Vermont).

Surgical Protocol

The rats were anesthetized throughout the procedures with 1.5% isoflurane (Abbott Laboratories, North Chicago, IL) and oxygen, and surgery was performed under sterile conditions. One 5-mm trocar (camera port) was introduced in the middle lower abdomen with an open technique, and pneumoperitoneum was created with carbon dioxide insufflation up to a pressure of 4 mm Hg. Two additional "ports" (2-mm trocars) were inserted in the left and right upper abdominal quadrant under direct vision **(Figure 1)**. Following gas insufflation and trocar placement, $1x10^7$ LMCR cells in 5 mL PBS were injected in the peritoneal cavity under laparoscopic vision through the 16-gauge needle attached to a 10 mL syringe. After 10 minutes, the two 2-mm trocars were removed and carbon dioxide insufflation was continued at a rate of 0.4 liters/minute and a pressure of 4 mm Hg for an additional 5

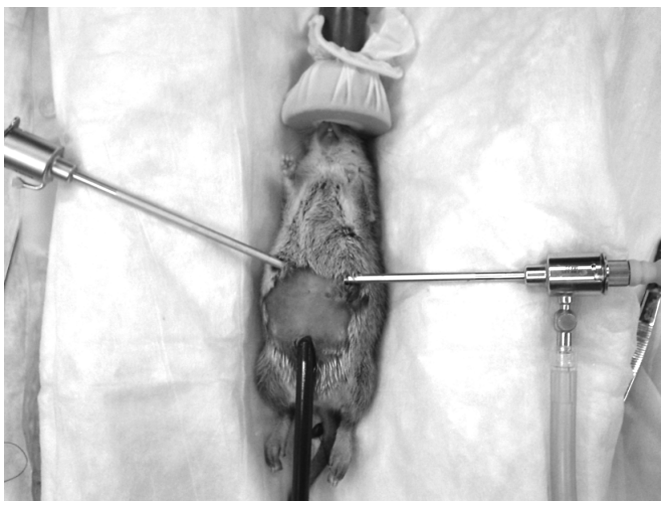

Figure 1. Pictures of the position of trocar placement.

minutes to allow the tumor cells to implant at the port sites. Oxaliplatin (0.198 mg/kg), 200 μL at a 500 μM concentration, was then applied topically via an intramuscular injection to one 2-mm trocar site (right side) by using a 27-gauge insulin syringe. The other port site was left untreated as the control. The ports were then closed with sutures. After 1 week, the rats were euthanized, and the abdomen was examined for the presence of tumor. The port sites were histologically examined for evidence of metastases.

Statistical Analysis

The frequency of port-site tumor development was compared between groups using the Fisher's exact probability test. Probabilities less than 0.05 were considered significant.

RESULTS

Cytotoxicity Following 1-Hour Exposure to Oxaliplatin

The cytotoxic effect of different concentrations of oxaliplatin is illustrated in **Figure 2**. All 3 cell numbers gave us the similar IC50 (1-hour exposure) values at 100-micromole oxaliplatin.

To determine the concentration of oxaliplatin with a 100% cytotoxic effect on LMCL (-) cells, we performed another in vitro study using oxaliplatin at 50 μm, 100 μm, and 500 μm concentrations. In the wells exposed to 500 μm, about 95% of the cells died. We therefore chose the 500 μM concentration of oxaliplatin (0.198 mg/kg) as our treatment dose.

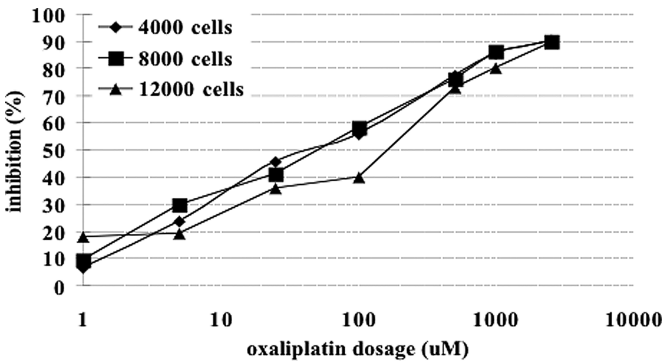

Figure 2. Inhibition of LMCR-dimethylhydrazine induced BDIX rat colonic adenocarcinoma on different oxaliplatin level.

Necropsy and Microscopic Findings

All rats survived for the duration of the study. During the autopsy, tumor was visible or palpable at the control port sites. All rats developed severe carcinomatosis in the abdominal cavity, including ascites in some cases. Macroscopically, the treated port site appeared to have a lower incidence of tumor nodule **(Figure 3)**. Microscopically, neoplastic cells were present individually or arranged in small aggregates or short cords. Neoplastic cells showed moderate to marked pleomorphism and had eosinophilic cytoplasm and normal to slightly hyperchromatic nuclei. Inflammation associated with tumor cells was generally mononuclear (plasma cells, lymphocytes, and few macrophages). In some areas, chronic active inflammation or granulomatous inflammation was seen. This was most likely due to the introduction of hairs into the tissue during the surgical procedure **(Figure 4)**.

Frequency of Port-Site Tumor Implantation

We observed that the number of animals with port-wound tumors was not significantly lower at the oxaliplatin treated port sites compared with the untreated group in the muscle layer (P=0.1) **(Figure 5)**. In the subcutaneous tissue, cancer cells were seen in 6 of 19 (32%) treated sites compared with 7 of 19 (37%) in the untreated ports (P=1). In the deep border skeletal muscle, cancer cells were seen in 13 of 19 (68%) treated ports and 14 of 19 (74%) untreated ports (P=1). Tumor growth was not significantly reduced in the subcutaneous tissue or the deep border skeletal muscle as a result of oxaliplatin treatment.

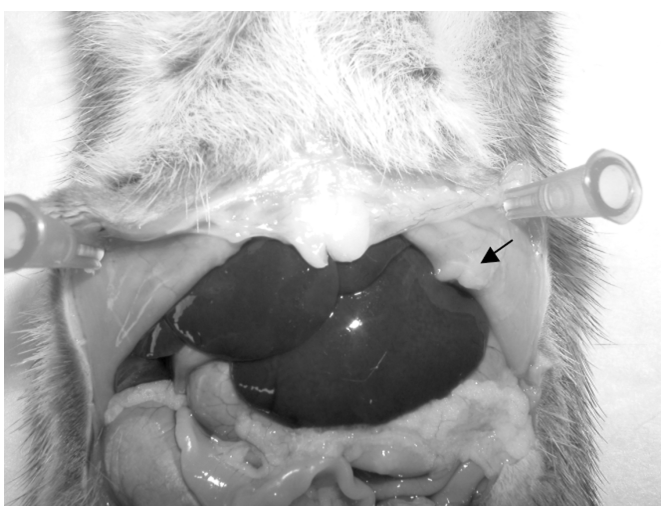

Figure 3. Gross findings during autopsy. Arrow indicated the port-site metastases.

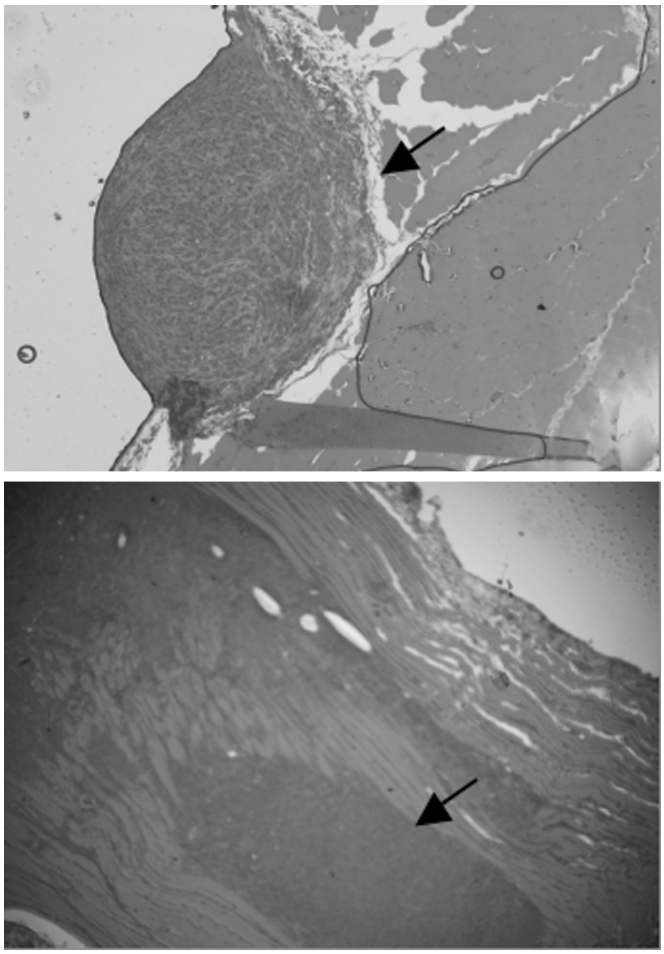

Figure 4. Upper panel (arrow) revealed the tumor cells in the peritoneum (100x H&E staining), lower panel (arrow) showed the tumor growth within the muscle (50x H&E staining).

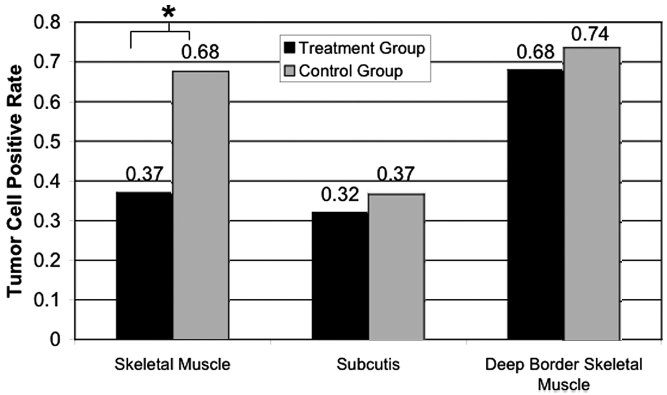

Figure 5. Port-site tumor incidence. *P=0.1.

DISCUSSON

The cause and real incidence of port-site tumor recurrences remain unknown, and therefore, efficient ways of prevention are still evolving. On anatomical grounds, it is unlikely that port tumors are the result of hematogenous or lymphatic spread. Direct implantation of tumor cells in the port-site or wound is the most likely mechanism.[16] Even though many surgeons still question the clinical significance of metastases arising in trocar wounds following laparoscopic intervention for malignancies, increasing evidence, both clinical and experimental, suggests a need for greater caution regarding its application to malignant growth.[13] It has been postulated that wound metastasis occurs as a result of contamination following laparoscopic manipulation of malignant tumors, with resultant spread to abdominal wall wounds by direct transfer when trocars and instruments are withdrawn from the peritoneal cavity.[22] In this instance, the development of barrier strategies to protect the wounds during laparoscopic surgery is supposed to be sufficient to overcome the problem. However, it is also possible that the insufflation gas used during laparoscopy mobilizes free cancer cells inside the abdominal cavity and thereby transfers them to the wounds without any direct physical contact with contaminated instruments. In our animal model, we were able to observe how it is possible for cancer cells to become implanted while the pneumoperitoneum is maintained after trocar removal. A variety of methods have been proposed to minimize the likelihood of port-site tumor implantation; ie, wound protectors, impermeable specimen retrieval bags, and cytotoxic or cytolytic irrigations,[11,17,23] but only one study has been performed to test the efficacy of direct injection of a cytotoxic agent. This study used intraperitoneal povidone-iodine and an appropriate dose of intraperitoneal or intramuscular methotrexate to demonstrate that a reduction in the incidence of port-site metastases might be achieved by the injection of appropriate tumoricidal agents.[15] In this study, IM methotrexate injection over the port site can reduce the number of port sites with tumor presentation from 74% to 39%, comparable with our result from 68% to 37%.

Oxaliplatin is a third-generation platinum derivative whose mechanism of action is similar to that of cisplatin. Oxaliplatin (l-OHP) differs from cisplatin by the presence of a diaminocyclohexane ligand in its chemical structure. This important difference in the molecule, and hence in the DNA adducts formed, confers a different spectrum of activity when compared with cisplatin.[24] Oxaliplatin is an active drug in the treatment of advanced colorectal carcinoma that is either chemotherapy naïve or refractory to 5-FU. In advanced colorectal cancer, 2 randomized studies

from Europe reported promising results with a combination of oxaliplatin, fluorouracil (FU), and leucovorin (LV) as first-line treatment compared with 5-FU and LV alone.[25,26] Oxaliplatin has been licensed in Europe since 1999, but it only gained FDA approval in the United States in August of 2002. Accordingly, we chose to study oxaliplatin as a possible cytotoxic agent to prevent port-site metastases. It is critical to determine the correct dosage of oxaliplatin to be used to enable the success of this modality of treatment. Therefore, we performed an in vitro study to test the cytotoxicity effect of this drug on the cancer cell line we used in our animal model and determine the concentration to be used in vivo. No necrosis was observed at the injection site based on the necropsy and our pathology findings. This proved that the dose of oxaliplatin chosen was not toxic to the tissues. The results of the current study cannot demonstrate a significant reduction in the incidence of tumor metastases in the treated muscle layer. There was no statistical significance between the treatment and control port sites (P=0.1). This suggests that the sample sizes were not enough or the dose of oxaliplatin that we applied in this study was too low. Nevertheless, we can still see some trend of declination of caner growth in the muscle layer. This suggests that probably an effective dose of oxaliplatin applied via an intramuscular injection may reduce the number of viable tumor cells in the muscle layer and prevent metastasis to develop from implanted viable cells into port-site wounds. The results also suggest that it is difficult to control cancer cell growth in all layers of the abdominal wall using a port-site injection of an anticancer drug. To solve this problem of drug delivery to other layers, the use of a biodegradable polymer[27] may be a more suitable alternative to injection.

CONCLUSION

Topical oxaliplatin might not be useful in reducing the incidence of port-site metastasis in our small number animal study. Nonetheless, further studies are needed to improve its method of application and dosage to verify whether this cytotoxic agent can be successfully applied to clinical practice.

References:

1. Stockdale AD, Pocock TJ. Abdominal wall metastasis following laparoscopy: a case report. *Eur J Surg Oncol.* 1985;11(4):373–375.

2. Ricardo AE, Feig BW, Ellis LM, et al. Gallbladder cancer and trocar site recurrences. *Am J Surg.* 1997;174(6):619–623.

3. Fusco MA, Paluzzi MW. Abdominal wall recurrence after laparoscopic-assisted colectomy for adenocarcinoma of the colon. Report of a case. *Dis Colon Rectum.* 1993;36(9):858–861.

4. Jacobs M, Verdeja JC, Goldstein HS. Minimally invasive colon resection (laparoscopic colectomy). *Surg Laparosc Endosc.* 1991;1(3):144–150.

5. Pappas TN. Laparoscopic colectomy—the innovation continues. *Ann Surg.* 1992;216(6):701–702.

6. Hoffman GC. Laparoscopic-assisted colectomy: initial experience. *Ann Surg.* 1994;219(6):732–743.

7. Franklin ME,Jr, Rosenthal D, Norem RF. Prospective evaluation of laparoscopic colon resection versus open colon resection for adenocarcinoma. A multicenter study. *Surg Endosc.* 1995;9(7):811–816.

8. Franklin ME, Kazantsev GB, Abrego D, Diaz-E JA, Balli J, Glass JL. Laparoscopic surgery for stage III colon cancer: long-term follow-up. *Surg Endosc.* 2000;14(7):612–616.

9. Lacy AM, Delgado S, Garcia-Valdecasas JC, et al. Port site metastases and recurrence after laparoscopic colectomy. A randomized trial. *Surg Endosc.* 1998;12(8):1039–1042.

10. Lacy AM, Garcia-Valdecasas JC, Delgado S, et al. Laparoscopy-assisted colectomy versus open colectomy for treatment of non-metastatic colon cancer: a randomised trial. *Lancet.* 2002;359(9325):2224–2229.

11. Eshraghi N, Swanstrom LL, Bax T, et al. Topical treatments of laparoscopic port sites can decrease the incidence of incision metastasis. *Surg Endosc.* 1999;13(11):1121–1124.

12. Paolucci V, Schaeff B, Schneider M, Gutt C. Tumor seeding following laparoscopy: international survey. *World J Surg.* 1999;23(10):989–997.

13. Mathew G, Watson DI, Ellis T, De Young N, Rofe AM, Jamieson GG. The effect of laparoscopy on the movement of tumor cells and metastasis to surgical wounds. *Surg Endosc.* 1997;11(12):1163–1166.

14. Martin JK Jr., Goellner JR. Abdominal fluid cytology in patients with gastrointestinal malignant lesions. *Mayo Clin Proc.* 1986;61(6):467–471.

15. Neuhaus SJ, Watson DI, Ellis T, Rofe AM, Jamieson GG. Influence of cytotoxic agents on intraperitoneal tumor implantation after laparoscopy. *Dis Colon Rectum.* 1999;42(1):10–15.

16. Lee SW, Gleason NR, Bessler M, Whelan RL. Peritoneal irrigation with povidone-iodine solution after laparoscopic-assisted splenectomy significantly decreases port-tumor recurrence in a murine model. *Dis Colon Rectum.* 1999;42(3):319–326.

17. Neuhaus SJ, Watson DI, Ellis T, Dodd T, Rofe AM, Jamieson GG. Efficacy of cytotoxic agents for the prevention of laparoscopic port-site metastases. *Arch Surg.* 1998;133(7):762–766.

18. Neuhaus SJ, Ellis T, Jamieson GG, Watson DI. Experimental study of the effect of intraperitoneal heparin on tumour implantation following laparoscopy. *Br J Surg*. 1999;86(3):400–404.

19. Balli JE, Franklin ME, Almeida JA, Glass JL, Diaz JA, Reymond M. How to prevent port-site metastases in laparoscopic colorectal surgery. *Surg Endosc*. 2000;14(11):1034–1036.

20. Boland CR. Colorectal cancer prevention and treatment. *Gastroenterology*. 2000;118(2):S115–S128.

21. Ogata S, Ho I, Maklansky J, et al. A rat model to study the role of STn antigen in colon cancer. *Glycoconj J*. 18(11–12):871–882, 2001.

22. Lee SW, Southall J, Allendorf J, Bessler M, Whelan RL. Traumatic handling of the tumor independent of pneumoperitoneum increases port site implantation rate of colon cancer in a murine model. *Surg Endosc*. 1998;12(6):828–834.

23. Martinez J, Targarona EM, Balague C, Pera M, Trias M. Port site metastasis. An unresolved problem in laparoscopic surgery. A review. *Int Surg*. 1995;80(4):315–321.

24. Chau I, Cunningham D. Oxaliplatin for colorectal cancer in the United States: better late than never. *J Clin Oncol*. 2003; 21(11):2049–2051.

25. de Gramont A, Figer A, Seymour M, et al. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol*. 2000;18(16):2938–2947.

26. Giacchetti S, Perpoint B, Zidani R, et al. Phase III multicenter randomized trial of oxaliplatin added to chronomodulated fluorouracil-leucovorin as first-line treatment of metastatic colorectal cancer. *J Clin Oncol*. 2000;18(1):136.

27. Lillehei KO, Kong Q, Withrow SJ, Kleinschmidt-DeMasters B. Efficacy of intralesionally administered cisplatin-impregnated biodegradable polymer for the treatment of 9L gliosarcoma in the rat. *Neurosurgery*. 1996;39(6):1191–1199.